PATENT SPECIFICATION (11) 1209326

1209326

NO DRAWINGS

(21) Application No. 42957/67 (22) Filed 21 Sept. 1967

(31) Convention Application No. 22135 (32) Filed 27 Sept. 1966 in

(33) Italy (IT)

(45) Complete Specification published 21 Oct. 1970

(51) International Classification C 07 d 49/18; A 61 k 27/00

(52) Index at acceptance

C2C 187—196—197 215 220 226 22Y 247 250 252 25Y 30Y 313 31Y 338 342 34Y 3A10E3D1 3A10E5E 3A14A3D 3A14A8A 574 579 584 594 62X 790 79Y KF

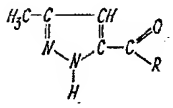
A5B 382 38Y 480 48Y 503 50Y 510 51Y 541 542 544 54Y 565 566 56Y 586 58Y 656 65Y 674 67Y

(54) THE PREPARATION OF 3-METHYL-5-PYRAZOLE CARBOXYLIC ACID AMIDES

(71) We, CARTER WALLACE, INC., a corporation organized and existing under the laws of the State of Maryland, United States of America of Two Park Avenue, New York, N.Y.10016, United States of America, do hereby declare the invention, for which we pray that a patent may be granted, to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

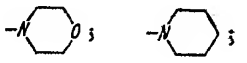
This invention relates to the preparation of 3 - methyl - 5 - pyrazolecarboxylic acid amides.

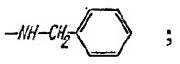
The products produced by the process of the invention have the following general formula:

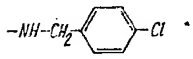


I

wherein R is NH_2 or a radical of a primary or secondary amine, such as







These compounds may be used in association with a pharmaceutical carrier in pharmaceutical compositions for the purposes described below.

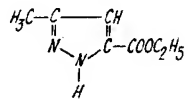
The amides of formula I are prepared according to the invention by a process comprising the steps of treating ethyl acetopyruvate, dissolved in glacial acetic acid, with hydrazine hydrate, cooling to cause 3 - methyl - 5 - pyrazolecarboxylic acid ethyl ester to precipitate, reacting said ester with ammonia or a primary or secondary amine in stoichiometrically equivalent proportions in an aqueous alcoholic solution, in an excess of the amine, or in aqueous solution containing an excess of the amine or ammonia, refluxing for 4 to 15 hours and collecting the precipitated amide. After the refluxing the amide is precipitated either by concentrationg or cooling the solution. The precipitate is recrystallised two or three times, as necessary.

The ethyl ester of the 3 - methyl - 5 - pyrazolecarboxylic acid is a known compound, described by Knorr (Annalen, *279*, page 219) of 1894 and the process of its preparation has been described in the German Patent No. 74,619.

This ester, however, when prepared by Knorr's method, that is the reaction of hydrazine sulphate with the sodium salt of ethyl acetopyruvate in the presence of NaOH in

aqueous solution, does not exhibit constant characteristics and so does not lend itself to the satisfactory preparation of the compounds of formula I.

The novel method for the preparation of the ethyl ester of 3 - methyl - 5 - pyrazolecarboxylic acid, having the formula:



used in this invention is, in more detail, as follows:

To 5 g. (0.032 mol) of ethyl aceto-pyruvate (prepared as disclosed in A. H. Blatt, "Organic Syntheses Coll. Vol. I, published by John Wiley & Sons, New York, 1958, page 233) dissolved in 5 mls. glacial acetic acid, 1.85 ml. of 85% hydrazine hydrate are added gradually and with stirring (0.032 mol.).

The solution is refluxed for 15—20 mins. and on cooling to room temperature, is poured into an ice-filled vessel. A flaky white precipitate is formed which is recrystallised from ligroin.

There are obtained 3.1 g. (yield 62%) of the ethyl ester 3 - methyl - 5 - pyrazolecarboxylic acid which is soluble in ligroin, poorly soluble in ethanol, diethyl ether and benzene, soluble in water only with difficulty. Melting point 81°C—82°C. (The chemical literature reports a m.p. of 82°—83°C.).

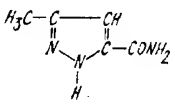
By changing either the primary or the secondary amine used in the preparation many compounds can be obtained which correspond to the general formula reported above.

Of the compounds of formula I the morpholide, piperidide, benzylamide, and *para*-chlorobenzylamide of 3 - methyl - 5 - pyrazolecarboxylic acid are novel and as such within the scope of the invention.

In the following Examples, a few of these compounds are described along with the procedure for their preparation, by way of example only and without any implied limitation thereto.

EXAMPLE 1

3 - methyl - 5 - pyrazolecarboxylic amide



6 g. (0.039 mol) of the ethyl ester of 3 - methyl - 5 - pyrazolecarboxylic acid are dissolved in the minimum possible amount of ethanol and then treated with an excess of aqueous ammonia.

The mixture is refluxed for 15 hours and a white solid precipitates on cooling the solid which is twice recrystallised from water, has one molecule of water of crystallisation. (Analysis: Calculated for $C_5H_7N_3O.H_2O$, N% = 29.34; Found: N% = 29.23).

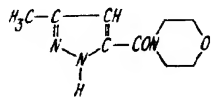
The product when heated for 6 hours in an oven under reduced pressure, loses its molecule of water. Yield 3.5 g. (63%). The desired anhydrous product melts at 174°C. and is poorly soluble in cold water and ethanol while it is fairly soluble in the same solvents when hot.

The product analyses:

For $C_5H_7N_3O$ Calculated: C% = 47.99; H% = 5.64; N% = 33.58
Found: C% = 47.79; H% = 5.61; N% = 33.15

EXAMPLE 2

3 - methyl - 5 - pyrazolecarboxylic acid morpholide



5 g. (0.032 mol) of the ethyl ester of 3 - methyl - 5 - pyrazolecarboxylic acid are refluxed with an excess of morpholine (10 ml. approx.) for about 4 hours. The mixture is diluted with water and then the water and morpholine are removed by heating under reduced pressure.

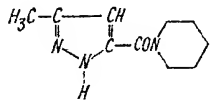
The residue thus obtained is recrystallised in the presence of decolorising carbon and dried in a vacuum oven. According to the best procedure, it is recrystallised twice from ethanol and precipitated with diethyl ether. There are obtained 3 g. (yield 50%) of the desired product as a white solid which melts at 188°C. It is soluble in water and ethanol, and insoluble in diethyl ether.

Analysis:

For $C_9H_{13}N_3O_2$ Calculated: C% = 55.37; H% = 6.71; N% = 21.52
Found: 55.67 6.67 21.43

EXAMPLE 3

3 - methyl - 5 - pyrazolecarboxylic acid piperidide



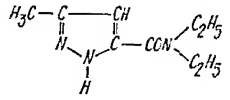
The same procedure as in Example 2 is followed except, instead of morpholine, an excess of piperidine (10 ml. approx.) is used. According to the best procedure, the product is twice recrystallised from aqueous ethanol. The desired product thus obtained (yield 53%) melts at 191°C—192°C. and is soluble in ethanol and poorly soluble in water.

The product analyses:

For $C_{10}H_{15}N_3O$ Calculated:	C% = 62.15;	H% = 7.82;	N% = 21.75
Found:	62.05	7.63	23.58

EXAMPLE 4

3 - methyl - 5 - pyrazolecarboxylic acid diethylamide



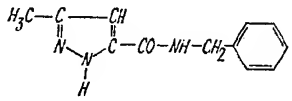
The same procedure as in Example 2 is followed except, instead of morpholine, an excess of diethylamine (10 ml. approx.) is used. According to the best procedure, the desired product is recrystallised twice from alcohol and precipitated with ether. A white solid (yield 53%) which melts at 164°C. and is soluble in water and ethanol, and insoluble in diethyl ether is obtained.

The product analyses:

For $C_9H_{15}N_3O$ Calculated:	C% = 59.64;	H% = 8.34;	N% = 23.18
Found:	59.36	8.12	23.21

EXAMPLE 5

3 - methyl - 5 - pyrazolecarboxylic acid benzylamide



5 g. (0.032 mol.) of the ethyl ester of 3 - methyl - 5 - pyrazolecarboxylic acid, dissolved in aqueous ethanol, are reacted with 3.3 g. of benzylamine by refluxing for 5 hours.

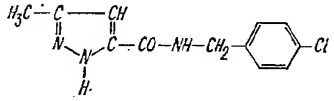
The solution is evaporated and the desired product thus obtained, when recrystallised twice from aqueous ethanol, melts at 116°C. and is soluble in ethanol, and insoluble in water. Yield 63%.

The product analyses:

For $C_{12}H_{13}N_3O$ Calculated:	C% = 66.95;	H% = 6.09;	N% = 19.52
Found:	67.42	6.06	19.67

EXAMPLE 6

3 - methyl - 5 - pyrazolecarboxylic acid - p - chlorobenzylamide



The procedure of Example 5 is followed by employing 4.3 g. of *p*-chlorobenzylamine and the precipitate thus obtained upon cooling is recrystallised twice from aqueous ethanol. The desired product melts at 202°C. and is poorly soluble in ethanol and insoluble in water. Yield 63%.

The product analyses:

For $C_{12}H_{12}ClN_3O$ Calculated:	C% = 57.72;	H% = 4.64;	N% = 16.83
Found:	58.20	4.69	16.61

All the compounds obtained according to the Examples from 1 to 6 are endowed with outstanding pharmacological properties such as an activity upon ketone bodies, lipids and triglycerides.

In the following the results of a pharmacological screening, aiming at ascertaining the activity of the 3 - methyl - 5 - pyrazolecarboxylic acid amide ($CONH_2$) are reported in comparison with 3,5 - dimethyl pyrazole (3.5 DMP).

Activity of 3 - methyl - 5 - pyrazolecarboxylic acid amide on the lipid metabolism.

Methods — Male Sprague-Dawley rats, 150 g. average weight, were used. In "*in vivo*" experiments 3,5-dimethylpyrazole (3.5 DMP) and 3 - methyl - 5 - pyrazolecarboxylic acid amide ($CONH_2$) were given to 18 hours fasted rats orally or intraperitoneally.

At the end of the experiment the levels of plasma free fatty acids (FFA), ketone bodies, triglycerides (TG), chloesterol (CHOL), phospholipids (P), of blood glucose and of liver triglycerides were determined.

FFA were determined according to Dole (J. Clin. Invest. 1956, *35*, 150) with minor modification. Lipids were extracted with a mixture of chloroform and methanol (2:1) and washed with saline. Phosphorus of phospholipids was determined in the chloroform extract according to Lowry *et al* (J. Biol. Chem. 1954, *207*, 1). The residual chloroform extracts were shaken with silicic acid and centrifuged. Cholesterol and triglycerides were determined in the supernatant liquid according to the Lieberman and Burchard reaction and to the Van Handel and Zilversmit method (J. Lab. Clin. Med. 1957, *50*, 153) respectively.

A further experiment was performed, during which rats were given $CONH_2$ each day for 15 days.

In other experiments $CONH_2$ and 3.5 DMP were given to rats treated with ethyl alcohol (1.6 ml./kg. *per os*).

Results — Table I gives the effect of $CONH_2$ on plasma FFA and ketone bodies. 18 hours fasted rats were given $CONH_2$ orally or intraperitoneally. Determinations were performed 30 min. or 60 min. after administration. $CONH_2$, given orally or intraperitoneally is effective in lowering plasma FFA and ketone bodies.

Table 2 gives the effect of $CONH_2$ and 3.5 DMP on plasma lipids, blood glucose and liver TG. The drugs were administered by oral route to 18 hour fasted rats and the determinations were performed 6 hr and 8 hours after the treatment. 6 hours after the administration the lowering effect of 3.5 DMP on plasma lipid is already ended while $CONH_2$ is still active. 8 hours after $CONH_2$ administration the plasma FFA levels rose again, but plasma and liver TG were still low.

The $CONH_2$ does not show any effect on blood cholesterol or phospholipids. Results in Tables 3 and 4 are concerned with a longer course of administration of $CONH_2$; the rats were divided in 2 groups. The first group received $CONH_2$ 7.5 mg./kg. by oral route every day for 15 days. The second group received saline. At the end of the treatment each group was further divided into 3 groups: the first one was fed, the second was fasted for 18 hours, the third received $CONH_2$ 1 hour before being killed. Results in Table 3 show that the growth rate of $CONH_2$ treated rats was the same as that of control rats, and also that the adipose tissue weight was not affected. Results in Table 4 show that the effect of a shorter course of administration of $CONH_2$ was not affected by the previous longer treatment.

Results in Table 5 show that $CONH_2$ as well as 3.5 DMP prevented the increase of liver TG induced by ethyl alcohol.

In Table 6 it is shown that only $CONH_2$, but not 3.5 DMP, is curative with respect to a fatty liver induced by alcohol.

In Summary — $CONH_2$ decreases FFA probably by blocking lipolysis in the adipose tissue. $CONH_2$ decreases plasma and liver TG and in addition it lowers the level of plasma, and ketone bodies. $CONH_2$ prevents and cures the fatty liver induced by a single dose of ethyl alcohol. 3.5 DMP, the reference drug, prevents but it does not cure this toxic effect of alcohol.

TABLE 1

Effect of $CONH_2$ on plasma FFA and ketone bodies on fasted rats

Treatment mg/kg		route	Time between treatment and killing	Plasma	
				FFA uEq/1	Ketone bodies mg/100 ml(°°)
Saline		—	—	699±18	14.0±0.5
$CONH_3$	1	i.p.	30′	235±24	8.5±0.6
Saline		—	—	554(°)	10.1±0.9
$CONH_2$	7.5	os	60′	143	4.2±0.5
	3.7	,,	,,	143	3.6±0.3
	1.7	,,	,,	172	4.2±0.1
	0.75	,,	,,	168	4.4±0.2
Saline		—	—	616(°)	15.-±2.-
$CONH_2$	0.75	os	60′	265	6.3±0.7
	0.37	,,	,,	386	5.8±0.6

(°°) determinations were performed on pooled sera.

(°) each figure is the average of at least 5 determinations.

TABLE 2

Effect of $CONH_2$ and 3.5 DMP on plasma lipids blood glucose and Liver TG.

Treatment mg/kg, oral	time between treatment and killing	PLASMA FFA uEq/l	PLASMA TG mg/100 ml	PLASMA CHOL mg/100 ml	PLASMA P mg/100 ml	Blood glucose mg/100 ml	Liver TG mg/100 ml
Saline	—	602±37	73±6	57±5	3.4±0.2	55±3	695±56
$CONH_2$ 7.5	6 hr	345±38	32±1	60±4	3.3±0.2	56+4	433±15
3.5 DMP 7.5	„	746±24	75±7	68±7	4.1±0.2	41±1	774±26
Saline	—	872±74	69±4			63±2	408±25
$CONH_2$ 7.5	6 hr	656±64	46±2			69±3	288±20
Saline	—	759±56	63±4			50±2	492±35
$CONH_2$ 7.5	6 hr	403±34	32±2			54±2	363±28
Saline	—	897±64	74±3			74±3	639±68
$CONH_2$ 7.5	8 hr	963±80	53±1			53±1	375±29
3.5 DMP 7.5	„	1098±52	58±2			58±2	579±49

Rats fasted 18 hr before the beginning of the experiment.

TABLE 3

Treatment mg/kg os	CONTROLS				$CONH_2$ 7.5 mg/kg os × 15 days			
			Blood cells				Blood cells	
	body wt. increase g	adipose tissue mg/b.w.	Red $10^6 mm^3$ ± S.E.	White $10^3 mm^3$ ± S.E.	body wt. increase g	adipose tissue mg/b.w.	Red 10^3 mm^3 ± S.E.	White 10^3 mm^3 ± S.E.
Fed	138	466	—	—	130	432	—	—
Fasted	92	386	6.42 ±0.09	14.7 ± 1.5	96	396	5.91 ± 0.4	12.8 ± 2.1
Fasted + CON_2 7.5	93	356	5.53 ±0.42	± 8.8 ± 1.7	84	411	6.06 ± 0.6	15.8 ± 6

TABLE 4

Treatment mg/kg os	CONTROLS					$CONH_2$ 7.5 mg/kg, os × 15 days				
	FFA uEq/1 ± E.E.	Glucose mg/100 ml ± S.E.	TG mg/100 ml ± S.E.	CHOL mg/100 ml ± S.E.	P mg/ml ±S.E.	uEq/1 ±S.E.	mg/100 ml ±S.E.	mg/100 ml ± S.E.	mg/100 ml ± S.E.	mg/ml ± S.E.
Fed	143 ±13	100 ±0.5	107 ± 4	50 ±6	4.1 ±0.05	155 ±25	93 ±3	109 ±7	74 ±7	5.2 ±0.2
Fasted	288 ±48	76 ±6	94 ±3	60 ±5	4.1 ±0.3	553 ±70	72 ±0.7	98 ±3	65 ±2	4.4 ±0.2
Fasted + $CONH_2$ 7.5	142 ± 25	68 ±7	63 ±10	61 ±4	3.9 ±0.4	271 ±47	74 ±3.9	72 ±7	59 ±2	3.9 ±0.3

TABLE 5

Preventive effect

Treatment	Liver TG mg/100 g
Saline	504
Alcohol 2.1 ml/kg, oral	1690
Alcohol + 3.5 DMP 15 mg/kg, i.p.	1026 (°)
Alcohol + $CONH_2$ 15 mg/kg, i.p.	721 (°)
Alcohol 1.6 ml/kg, oral	1161
Alcohol + $CONH_2$ 15 mg/kg i.p.	552 (°)

Animals were fasted for 18 hrs. Drugs were given together with alcohol (50+ solution). Animals were killed 8 hr after the end of the treatment.

(°) P 0.01 in respect to controls.

TABLE 6

Curative effect

Treatment	Liver TG mg/100 g.
Saline	486
Alcohol 1.6 ml/kg, oral	1180
Alcohol + 3.5 DMP 15 mg/kg, i.p	1200
Alcohol + $CONH_2$ 15 mg/kg, i.p.	867 (°)

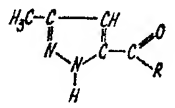
Animals were fasted for 18 hrs Drugs were given 1 hr before killing. Alcohol was given 8 hr before killing.

(°) P 0.01

When employed as anti-lipaemic agents, the compounds of formula I are preferably administered via the oral route in the form of dragées, tabloids, capsules, syrups, or elixirs. The compounds can also be administered by injection by employing a suspension of the compound in water or physiological saline, or an aqueous solution thereof or a solution of the compound concerned in a solvent consisting of aqueous propylene glycol, dimethyl guanide, sodium salicylate, methyl glucosamine or aqueous polyethylene glycol. In addition to the active ingredients, the tabloids contain fillers, extenders, lubricants and so forth of any conventional kind. Generally the active ingredient is from 25% to 90% of the total composition, by weight. Typical examples of said tabloids or capsules are those containing from 20 to 200 milligrams of active ingredient, preferably 100 milligrams.

WHAT WE CLAIM IS:—

1. A process for preparing amides of 3 - methyl - 5 - pyrazolecarboxylic acid having the general formula:



wherein R is NH_2 or a radical of either a primary or a secondary amine, comprising the steps of treating ethyl acetopyruvate, dissolved in glacial acetic acid, with hydrazine hydrate, cooling to cause 3 - methyl - 5 - pyrazolecarboxylic acid ethyl ester to precipitate, reacting said ester with ammonia or a primary or secondary amine in stoichiometrically equivalent proportions in an aqueous alcoholic solution, in an excess of the amine, or in aqueous solution containing an excess of the amine or ammonia, refluxing for 4 to 15 hours and collecting the precipitated amide.

2. A process according to claim 1, wherein the ethyl ester of 3 - methyl - 5 - pyrazolecarboxylic acid is treated with ammonia, morpholine, piperidine, benzylamine or *para*-chlorobenzylamine.

3. A process according to claim 1 substantially as described.

4. An amide of 3 - methyl - 5 - pyrazolecarboxylic acid when produced by the process of any of claims 1 to 3.

5. The morpholide of 3 - methyl - 5 - pyrazolecarboxylic acid.

6. The piperidine of 3 - methyl - 5 - pyrazolecarboxylic acid.

7. The benzylamide of 3 - methyl - 5 - pyrazolecarboxylic acid.

8. The *para* - chlorobenzylamide of 3 - methyl - 5 - pyrazolecarboxylic acid.

9. A pharmaceutical composition comprising, in association with a pharmaceutical carrier, an amide as claimed in any one of claims 4 to 8.

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Reference has been directed in pursuance of Section 9, Subsection (1) of the Patents Act, 1949, to patent No. 1,048,104.

Printed for Her Majesty's Stationery Office by the Courier Press, Leamington Spa, 1970.
Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.